肺癌多基因功能分析及调控网络模型的建立

李　璐[1],谷建钟[2]

(1.郑州大学附属肿瘤医院,河南 郑州 450003;2.浙江省中医医院,浙江 杭州 310006)

摘　要:[目的]探讨肺癌多基因的功能,并建立肺癌细胞增殖调控网络模型。[方法]基于前期对MMP-7蛋白交联作用分析,利用基因本体论(Gene Ontology,GO)和基因组百科全书数据库(Kyoto Encyclopedia of Genes and Genomes,KEGG),对与之具有交联作用的*ZBTB33*、*NGF*、*COL18A1*、*MMP-1*、*TIMP1*、*CTNNB1*、*JUP*、*CD44*、*SDC1*、*HBEGF*进行功能分析和肺癌细胞增殖调控网络模型的建立。[结果]综合GO功能分析和KEGG分析结果,初步建立肺癌细胞增殖调控网络模型,并分析调控网络模型中基因的作用。[结论]成功构建上述基因在肺癌细胞增殖中的调控网络模型,为进一步研究肺癌发病机制及其治疗提供一定的理论基础。

主题词:肺肿瘤;MMP-7;功能分析;调控网络模型;细胞增殖

中图分类号:R734.2　　**文献标识码**:A　　**文章编号**:1671-170X(2018)06-0611-010

doi:10.11735/j.issn.1671-170X.2018.06.B016

Functional Analysis of Multiple Genes in Lung Cancer and Establishment of Regulatory Network

LI Lu[1],GU Jiang-zhong[2]

(1. *Affiliated Cancer Hospital of Zhengzhou University*,*Zhengzhou* 450003,*China*;2. *Zhejiang Provincial Hospital of TCM*,*Hangzhou* 310006,*China*)

Abstract:[Objective] To investigate the function of multiple genes in lung cancer and establish proliferation regulatory network of lung cancer cells. [Methods] Based on previous analysis of MMP-7's cross linking,Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) were used to conduct functional analysis for genes cross linked to MMP-7,including ZBTB33, NGF,COL18A1,MMP-1,TIMP1,CTNNB1,JUP,CD44,SDC1,HBEGF,and to establish proliferation regulatory network. [Results] After synthesizing results of GO and KEGG analysis,proliferation regulatory network of lung cancer cells was preliminarily established and the roles that genes played in the network were analyzed. [Conclusion] The proliferation regulatory network of lung cancer cells including the above genes was successfully established,providing theoretical foundation for further study in pathogenesis of lung cancer and its treatment.

Subject words:lung cancer;MMP-7;functional analysis;regulatory network;cell proliferation

肺癌是最常见的呼吸系统恶性肿瘤之一,在我国肺癌的发病率和死亡率均呈上升趋势,并随着我国工业化速度的加快、环境污染加重、人口老龄化加剧,肺癌的癌症负担日趋加重[1]。非小细胞肺癌是肺癌最常见的类型,占肺癌的85%,但70%的患者在确诊时已是晚期,且5年生存率仅为15%,如果早期治疗,5年生存率可达到85%[2]。基质金属蛋白酶-7(matrix metalloproteinase-7,MMP-7)是细胞外基质(extracellular matrix,ECM)溶解最主要的酶[3],具有广泛的底物特异性和强大的基质降解能力,并能通过刺激血管内皮细胞DNA合成来促进血管生成和肿瘤细胞的转移[4-6]。在生理或病理条件下,MMP-7可以通过与MMP-1、MMP-3、MMP-9的相互作用共同调节ECM的降解,MMP-3能够激活Pro MMP-7,而MMP-7又能增加MMP-1、MMP-9的活性。大量研究结果表明,MMP-7的表达与前列腺癌[7]、大肠癌[8]、乳腺癌[9]、宫颈癌[10]、直肠癌[11]等肿瘤的发生和转移密切相关。MMP-7受原癌基因、生长因子、激素和一些细胞因子的调节,但其调控机制尚不清楚[12]。

基金项目:浙江省科技厅公益技术项目(2015C33172)

通讯作者:李璐,主治医师,硕士;郑州大学附属肿瘤医院中西医结合肿瘤内科,河南省郑州市东明路127号(450003);E-mail:li_lu_10@sina.com

收稿日期:2017-01-16

利用基因本体论(Gene Ontology,GO)、美国国立生物技术信息中心 (National Center for Biotechnology Information,NCBI)、DNA 序列数据库(GenBank)、基因组百科全书数据库(Kyoto Encyclopedia of Genes and Genomes,KEGG) 等生物信息学数据库，寻找肿瘤基因标志物并构建复杂的基因调控网络模型，通过该模型来描述基因表达和调控之间的关系，使得可以通过基因调控网络模型研究肿瘤的发生发展成为可能。本研究基于课题前期利用 STRING 9.0 交互式数据库发现的与 MMP-7 具有交联作用的蛋白(ZBTB33、COL18A1、NGF、MMP-1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF)，进行 GO 分析以及 KEGG 分析，并构建这些基因在肺癌细胞增殖调控网络模型，为进一步研究肺癌的发病机制奠定基础。

1 材料与方法

1.1 研究对象

MMP-7 交联蛋白 ZBTB33、NGF、COL18A1、MMP1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF。

1.2 GO 分析

进入 AmiGO 的首页(http://geneontology.org)，输入 ZBTB33、NGF、COL18A1、MMP1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF,分别对其进行 GO 分析。

1.3 KEGG 分析

进入 KEGG 信号通路数据库首页(http://www.kegg.jp/kegg/pathway.html)，筛选条件 organism 为“hsa”,key words 为 ZBTB33、NGF、COL18A1、MMP1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF。

2 结 果

2.1 基因的 GO 分析

GO 分析的分类依据是基因编码的蛋白质在细胞中的作用,GO 分析结果涵盖生物过程 (biological process)、细胞组分(cellular component)和分子功能(molecular function)共三大部分。*ZBTB33* 基因 GO 分析结果如 Table 1 所示，ZBTB33 具有生物过程、细胞组分和分子功能共 3 种功能。ZBTB33 生物过程主要涉及以 DNA 为模板的转录、Wnt 信号通路、细胞内信号转导以及对以 DNA 为模板转录的负调控;细胞组分涉及细胞核中的核、核仁、细胞质和质膜;分子功能包括蛋白质结合、甲基-CpG 结合和与特异性与 DNA 序列结合。

NGF 基因的 GO 分析结果如 Table 2 所示,NGF 具有生物过程、细胞组分、分子功能 3 种功能。生物过程主要涉及 MAPKK 活性的激活、半胱氨酸内肽酶活性的激活、微管运动、细胞信号、跨膜受体蛋白酪氨酸激酶标志、调节基因表达、调节神经元分化;细胞组分包括胞外区；分子功能包括神经生长因子受体结合、蛋白结合、生长因子活性、金属内肽酶抑

Table 1 GO analysis of *ZBTB33* gene

Name	Ontology	GO Number
Transcription, DNA-templated	Biological_process	GO:0006351
Wnt signaling pathway	Biological_process	GO:0016055
Intracellular signal transduction	Biological_process	GO:0035556
Negative regulation of transcription, DNA-templated	Biological_process	GO:0045892
Nucleus	Cellular_component	GO:0005634
Nucleolus	Cellular_component	GO:0005730
Cytoplasm	Cellular_component	GO:0005737
Plasma membrane	Cellular_component	GO:0005886
Protein binding	Molecular_function	GO:0005515
Methyl-CpG binding	Molecular_function	GO:0008327
Sequence-specific DNA binding	Molecular_function	GO:0043565

Table 2 GO analysis of *NGF* gene

Name	Ontology	GO Number
Activation of MAPKK activity	Biological_process	GO:0000186
Activation of cysteine-type endopeptidase activity	Biological_process	GO:0006919
Microtubule-based movement	Biological_process	GO:0007018
Transmembrane receptor protein tyrosine kinase sign	Biological_process	GO:0007169
Cell-cell signaling	Biological_process	GO:0007267
Extrinsic apoptotic signaling pathway via death dom	Biological_process	GO:0008625
Positive regulation of gene expression	Biological_process	GO:0010628
Regulation of neuron differentiation	Biological_process	GO:0045664
Extracellular region	Cellular_component	GO:0005576
Nerve growth factor receptor binding	Molecular_function	GO:0005163
Protein binding	Molecular_function	GO:0005515
Growth factor activity	Molecular_function	GO:0008083
Metalloendopeptidase inhibitor activity	Molecular_function	GO:0008191

制剂活性。

COL18A1 基因的 GO 分析结果如 Table 3 所示，COL18A1 具有生物过程细胞组分、分子功能。生物过程涉及血管生成、内皮细胞形态发生及凋亡、细胞黏附、视觉感知、细胞增殖、器官形态的发生、ECM 组织、细胞迁移、胶原代谢分解以及对药物及静水压力的响应；细胞组分包括胶原三聚体、基底膜、胞外空间、内质网腔、ECM 以及胞外外体等；分子功能包括结构分子活性、蛋白结合、相同蛋白结合以及金属离子结合。

MMP-1 基因的 GO 分析结果如 Table 4 所示，MMP-1 具有生物过程、细胞组分和分子功能。生物过程涉及蛋白水解、病毒过程、ECM 拆卸、胶原分解代谢过程、蛋白低聚的正调控、细胞蛋白代谢过程和白细胞迁移；分子功能包括内金属内肽酶及丝氨酸内肽酶活性、钙离子结合和锌离子结合。

TIMP1 基因的 GO 分析结果如 Table 5 所示，TIMP1 具有生物过程、细胞组分和分子功能。生物过程涉及血小板脱颗粒、老化、细胞增殖、反应激素、内肽酶活性的负调节、ECM 的拆卸、细胞因子的应答、伤口愈合、调节凋亡过程及催化活性、软骨发育和调节整合素介导的信号通路；细胞组分包括胞外区、胞外基质蛋白、基底膜、胞外空间、血小板 α 颗粒腔和胞外外体；分子功能涉及蛋白酶的结合、细胞因子活性、蛋白结合、生长因子活性、金属内肽酶抑制剂活性和金属离子结合。

CTNNB1 基因的 GO 分析结果如 Table 6 所示，CTNNB1 具有生物过程、细胞组分和分子功能 3 种功能。生物过程包括细胞分化方向的调控、正调节神经细胞的增殖、正调节间充质细胞的增殖、负调节细胞增殖、Wnt 信号通路、正调节凋亡过程、正调节 I-κB 激酶/ NF-κB 信号和调节血管生成；细胞组分包括核、转录因子复合物、细胞质、质膜、Wnt 信号体；

Table 3　GO analysis of *COL18A1* gene

Name	Ontology	GO Number
Angiogenesis	Biological_process	GO:0001525
Endothelial cell morphogenesis	Biological_process	GO:0001886
Cell adhesion	Biological_process	GO:0007155
Visual perception	Biological_process	GO:0007601
Positive regulation of cell proliferation	Biological_process	GO:0008284
Negative regulation of cell proliferation	Biological_process	GO:0008285
Animal organ morphogenesis	Biological_process	GO:0009887
Extracellular matrix organization	Biological_process	GO:0030198
Positive regulation of cell migration	Biological_process	GO:0030335
Collagen catabolic process	Biological_process	GO:0030574
Response to drug	Biological_process	GO:0042493
Response to hydrostatic pressure	Biological_process	GO:0051599
Positive regulation of endothelial cell apoptotic p	Biological_process	GO:2000353
Extracellular region	Cellular_component	GO:0005576
Collagen trimer	Cellular_component	GO:0005581
Basement membrane	Cellular_component	GO:0005604
Extracellular space	Cellular_component	GO:0005615
Endoplasmic reticulum lumen	Cellular_component	GO:0005788
Extracellular matrix	Cellular_component	GO:0031012
Extracellular exosome	Cellular_component	GO:0070062
Structural molecule activity	Molecular_function	GO:0005198
Protein binding	Molecular_function	GO:0005515
Identical protein binding	Molecular_function	GO:0042802
Metal ion binding	Molecular_function	GO:0046872

Table 4　GO analysis of *MMP-1* gene

Name	Ontology	GO Number
Proteolysis	Biological_process	GO:0006508
Viral process	Biological_process	GO:0016032
Extracellular matrix disassembly	Biological_process	GO:0022617
Collagen catabolic process	Biological_process	GO:0030574
Positive regulation of protein oligomerization	Biological_process	GO:0032461
Cellular protein metabolic process	Biological_process	GO:0044267
Leukocyte migration	Biological_process	GO:0050900
Extracellular region	Cellular_component	GO:0005576
Proteinaceous extracellular matrix	Cellular_component	GO:0005578
Endopeptidase activity	Molecular_function	GO:0004175
Metalloendopeptidase activity	Molecular_function	GO:0004222
Serine-type endopeptidase activity	Molecular_function	GO:0004252
Calcium ion binding	Molecular_function	GO:0005509
Zinc ion binding	Molecular_function	GO:0008270

分子功能包括信号传感器活性、蛋白结合、转录因子结合、转录调节区 DNA 结合和离子通道结合。

JUP 基因的 GO 分析结果如 Table 7 所示，JUP 有生物过程、细胞组分和分子功能 3 种功能。其中生物过程涉及信号转导、细胞迁移、细胞增殖的调节、蛋白导入核的正向调节、正调节与特异性 DNA 序列结合的转录因子活性、内皮细胞-细胞黏附和 Wnt 信号通路的正调节；细胞组分包括核、细胞质、细胞骨架、质膜、连环蛋白复合物、ECM、蛋白质-DNA 复合物核胞外外体等；分子功能包括转录共激活剂活性、结构分子活性、蛋白结合、α-连环蛋白结合、细胞黏附分子结合和钙黏蛋白参与的细胞间黏附等。

CD44 基因的 GO 分析结果如 Table 8 所示，CD44 具有生物过程、细胞组分和分子功能 3 种功能。生物过程涉及细胞间质黏附、单一生物体细胞-细胞黏附、ECM 拆卸、透明质酸分解代谢过程、正调节激酶活性、负调节凋亡过程及半胱氨酸内肽酶活性、DNA 损伤的负调节和 p53 类蛋白介导的信号转导、白细胞迁移、软骨发育和 ERK1/ERK2 级联的正调节；细胞组分包括细胞质、质膜、质膜的整体组分、细胞表面、巨噬细胞迁移抑制因子受体复合物和胞外外体等；分子功能包括细胞因子受体活性和透明质酸结合等。

Table 5　GO analysis of *TIMP1* gene

Name	Ontology	GO Number
Platelet degranulation	Biological_process	GO:0002576
Aging	Biological_process	GO:0007568
Positive regulation of cell proliferation	Biological_process	GO:0008284
Response to hormone	Biological_process	GO:0009725
Negative regulation of endopeptidase activity	Biological_process	GO:0010951
Extracellular matrix disassembly	Biological_process	GO:0022617
Response to cytokine	Biological_process	GO:0034097
Wound healing	Biological_process	GO:0042060
Negative regulation of apoptotic process	Biological_process	GO:0043066
Negative regulation of catalytic activity	Biological_process	GO:0043086
Response to peptide hormone	Biological_process	GO:0043434
Negative regulation of membrane protein ectodomain	Biological_process	GO:0051045
Cartilage development	Biological_process	GO:0051216
Negative regulation of trophoblast cell migration	Biological_process	GO:1901164
Regulation of integrin-mediated signaling pathway	Biological_process	GO:2001044
Extracellular region	Cellular_component	GO:0005576
Proteinaceous extracellular matrix	Cellular_component	GO:0005578
Basement membrane	Cellular_component	GO:0005604
Extracellular space	Cellular_component	GO:0005615
Platelet alpha granule lumen	Cellular_component	GO:0031093
Extracellular exosome	Cellular_component	GO:0070062
Protease binding	Molecular_function	GO:0002020
Cytokine activity	Molecular_function	GO:0005125
Protein binding	Molecular_function	GO:0005515
Growth factor activity	Molecular_function	GO:0008083
Metalloendopeptidase inhibitor activity	Molecular_function	GO:0008191
Metal ion binding	Molecular_function	GO:0046872

Table 6　GO analysis of *CTNNB1* gene

Name	Ontology	GO Number
Cell fate specification	Biological_process	GO:0001708
Positive regulation of neuroblast proliferation	Biological_process	GO:0002052
Positive regulation of mesenchymal cell proliferation	Biological_process	GO:0002053
Negative regulation of cell proliferation	Biological_process	GO:0008285
Wnt signaling pathway	Biological_process	GO:0016055
Positive regulation of apoptotic process	Biological_process	GO:0043065
Positive regulation of I-kappaB kinase/NF-kappaB signaling	Biological_process	GO:0043123
Regulation of angiogenesis	Biological_process	GO:0045765
Nucleus	Cellular_component	GO:0005634
Transcription factor complex	Cellular_component	GO:0005667
Cytoplasm	Cellular_component	GO:0005737
Plasma membrane	Cellular_component	GO:0005886
Wnt signalosome	Cellular_component	GO:1990909
Signal transducer activity	Molecular_function	GO:0004871
Protein binding	Molecular_function	GO:0005515
Transcription factor binding	Molecular_function	GO:0008134
Transcription regulatory region DNA binding	Molecular_function	GO:0044212
Ion channel binding	Molecular_function	GO:0044325

Table 7　GO analysis of *JUP* gene

Name	Ontology	GO Number
Signal transduction	Biological_process	GO:0007165
Cell migration	Biological_process	GO:0016477
Regulation of cell proliferation	Biological_process	GO:0042127
Positive regulation of protein import into nucleus	Biological_process	GO:0042307
Positive regulation of sequence-specific DNA binding transcription factor activity	Biological_process	GO:0051091
Endothelial cell-cell adhesion	Biological_process	GO:0071603
Positive regulation of canonical Wnt signaling pathway	Biological_process	GO:0090263
Nucleus	Cellular_component	GO:0005634
Cytoplasm	Cellular_component	GO:0005737
Cytoskeleton	Cellular_component	GO:0005856
Plasma membrane	Cellular_component	GO:0005886
Catenin complex	Cellular_component	GO:0016342
Extracellular matrix	Cellular_component	GO:0031012
Protein-DNA complex	Cellular_component	GO:0032993
Extracellular exosome	Cellular_component	GO:0070062
Transcription coactivator activity	Molecular_function	GO:0003713
Structural molecule activity	Molecular_function	GO:0005198
Protein binding	Molecular_function	GO:0005515
Alpha-catenin binding	Molecular_function	GO:0045294
Cell adhesion molecule binding	Molecular_function	GO:0050839
Cadherin binding involved in cell-cell adhesion	Molecular_function	GO:0098641

Table 8　GO analysis of *CD44* gene

Name	Ontology	GO Number
Cell-matrix adhesion	Biological_process	GO:0007160
Single organismal cell-cell adhesion	Biological_process	GO:0016337
Extracellular matrix disassembly	Biological_process	GO:0022617
Hyaluronan catabolic process	Biological_process	GO:0030214
Positive regulation of kinase activity	Biological_process	GO:0033674
Negative regulation of apoptotic process	Biological_process	GO:0043066
Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	Biological_process	GO:0043154
Negative regulation of DNA damage response, signal transduction by p53 class mediator	Biological_process	GO:0043518
Leukocyte migration	Biological_process	GO:0050900
Cartilage development	Biological_process	GO:0051216
Positive regulation of ERK1 and ERK2 cascade	Biological_process	GO:0070374
Cytoplasm	Cellular_component	GO:0005737
Plasma membrane	Cellular_component	GO:0005886
Integral component of plasma membrane	Cellular_component	GO:0005887
Cell surface	Cellular_component	GO:0009986
Macrophage migration inhibitory factor receptor complex	Cellular_component	GO:0035692
Extracellular exosome	Cellular_component	GO:0070062
Cytokine receptor activity	Molecular_function	GO:0004896
Hyaluronic acid binding	Molecular_function	GO:0005540

SDC1 基因的 GO 分析结果如 Table 9 所示，SDC1 具有生物过程、细胞组分和分子功能。生物过程涉及炎症反应、对有毒物质的反应、细胞迁移、伤口愈合、对 cAMP 的反应和 Wnt 信号通路等；细胞组分涉及细胞质、高尔基体腔、质膜、焦点黏附、质膜外侧、细胞表面和溶酶体腔等；分子功能涉及糖蛋白

结合、蛋白结合和蛋白 C-末端结合等。

HBEGF 基因 GO 分析结果如 Table 10 所示,HBEGF 有生物过程、细胞组分和分子功能 3 种功能。生物过程涉及 MAPK 级联、表皮生长因子受体信号通路、正调节细胞增殖、调节磷酸酯肌醇 3-激酶信号、正调节细胞生长及迁移、ERBB2 信号通路、磷脂酰肌醇介导的信号通路、正调节平滑肌细胞增殖、负调节弹性蛋白生物合成过程和蛋白激酶 B 信号的正调节等;细胞组分涉及胞外区、质膜、质膜的整体组分和细胞表面等;分子功能涉及蛋白酪氨酸激酶活性、表皮生长因子受体结合、生长因子活性、肝素结合和磷脂酰肌醇-4,5-二磷酸 3-激酶活性等。

Table 9　GO analysis of *SDC1* gene

Name	Ontology	GO Number
Inflammatory response	Biological_process	GO:0006954
Response to toxic substance	Biological_process	GO:0009636
Cell migration	Biological_process	GO:0016477
Wound healing	Biological_process	GO:0042060
Response to cAMP	Biological_process	GO:0051591
Canonical Wnt signaling pathway	Biological_process	GO:0060070
Cytoplasm	Cellular_component	GO:0005737
Golgi lumen	Cellular_component	GO:0005796
Plasma membrane	Cellular_component	GO:0005886
Focal adhesion	Cellular_component	GO:0005925
External side of plasma membrane	Cellular_component	GO:0009897
Cell surface	Cellular_component	GO:0009986
Lysosomal lumen	Cellular_component	GO:0043202
Glycoprotein binding	Molecular_function	GO:0001948
Protein binding	Molecular_function	GO:0005515
Protein C-terminus binding	Molecular_function	GO:0008022

Table 10　GO analysis of *HBEGF* gene

Name	Ontology	GO Number
MAPK cascade	Biological_process	GO:0000165
Epidermal growth factor receptor signaling pathway	Biological_process	GO:0007173
Positive regulation of cell proliferation	Biological_process	GO:0008284
Regulation of phosphatidylinositol 3-kinase signaling	Biological_process	GO:0014066
Positive regulation of cell growth	Biological_process	GO:0030307
Positive regulation of cell migration	Biological_process	GO:0030335
ERBB2 signaling pathway	Biological_process	GO:0038128
Phosphatidylinositol-mediated signaling	Biological_process	GO:0048015
Positive regulation of smooth muscle cell proliferation	Biological_process	GO:0048661
Negative regulation of elastin biosynthetic process	Biological_process	GO:0051545
Positive regulation of protein kinase B signaling	Biological_process	GO:0051897
Positive regulation of wound healing	Biological_process	GO:0090303
Extracellular region	Cellular_component	GO:0005576
Plasma membrane	Cellular_component	GO:0005886
Integral component of plasma membrane	Cellular_component	GO:0005887
Cell surface	Cellular_component	GO:0009986
Protein tyrosine kinase activity	Molecular_function	GO:0004713
Epidermal growth factor receptor binding	Molecular_function	GO:0005154
Growth factor activity	Molecular_function	GO:0008083
Heparin binding	Molecular_function	GO:0008201
Phosphatidylinositol-4,5-bisphosphate 3-kinase activity	Molecular_function	GO:0046934

2.2　肺癌多基因调控网络模型的建立

在 GO 分析的基础上,利用 KEGG 数据库对本研究所选 *MMP-7*、*ZBTB33*、*NGF*、*COL18A1*、*MMP1*、*TIMP1*、*CTNNB1*、*JUP*、*CD44*、*SDC1*、*HBEGF* 基因进行 KEGG 分析,并成功建立这些基因参与的肺癌细胞增殖调控网络,结果如 Figure 1 所示。ZBTB33 可以通过 p53 信号通路促进肿瘤细胞的增殖;JUP 通过癌症中的信号通路促进肿瘤细胞的增殖;MMP-1、MMP-7 和 TIMP1 在正常的细胞及组织中保持一定的平衡,当平衡遭到破坏以后影响肿瘤细胞的增殖;SDC1 通过 Wnt/β-catenin 信号通路及细胞因子及其受体的相互作用来调控肿瘤细胞的增殖;HBEGF 和 NGF 通过细胞因子与受体的相互作用调控肿瘤细胞的增殖;β-catenin 通过 Wnt/β-catenin 信号通路调控肿瘤细胞的增殖,并能上调 *MMP-7* 基因的上调表达来调节肿瘤细胞的增殖;CD44 通过 p53 类蛋白介导的信号转导及 ERK1/ERK2 级联反应来实现对肿瘤细胞增殖的调控作用。通过查阅相关文献,发现 COL18A1 蛋白产生的内皮抑制素能够与细胞核表面的核仁蛋白

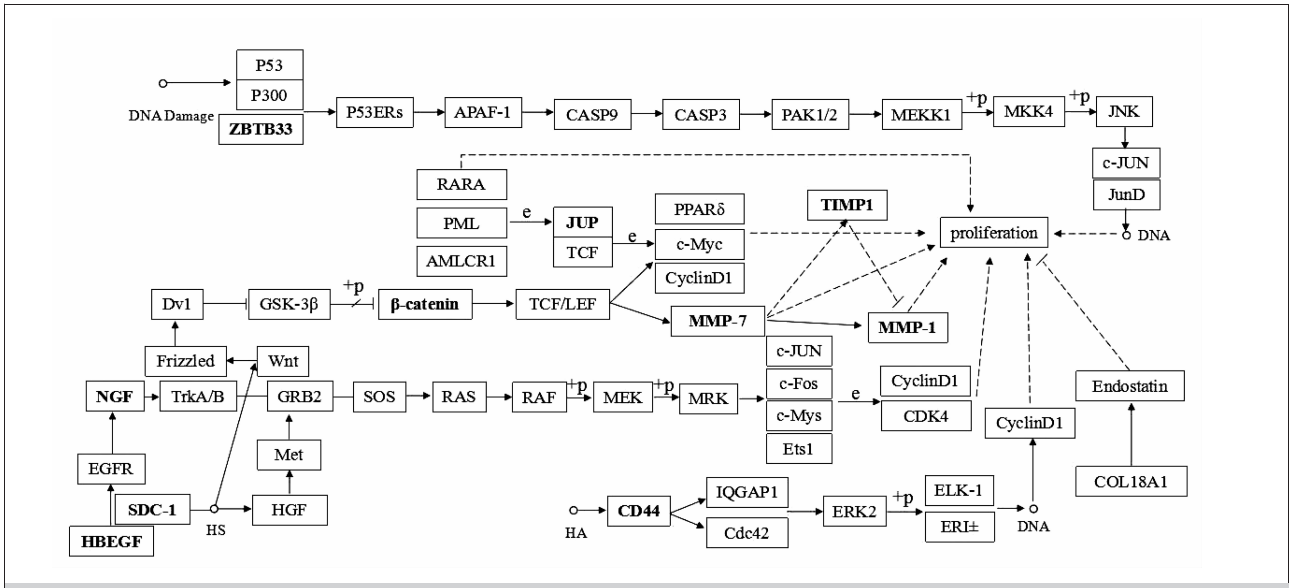

Figure 1　Cell proliferation regulatory network

结合，引起细胞生长周期的阻滞及凋亡，故将COL18A1补充至该调控网络中，但其参与的信号网络调控的具体机制等内容还不清楚，有待深入研究。

3　讨　论

肺癌的发生、发展是一个极其复杂的生理过程，涉及多种基因和蛋白的表达异常，并且由于恶性肿瘤的侵袭性强、易转移、生长快等特点，早期的诊断方法及治疗技术亟待改进。分子靶向药物具有特异性抑制肿瘤细胞信号转导通路的功能，治疗肿瘤的优势较为明显，可以有选择性的抑制肿瘤细胞，而对患者体内的正常细胞无任何毒杀作用，不影响正常细胞的生长及分化。本课题前期利用STRING 9.0交互式数据库进行蛋白质交联分析，发现在与*MMP-7*基因有作用的蛋白质中主要有ZBTB33、NGF、COL18A1、MMP1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF[13](Figure 2)。本研究在此基础上进行GO分析及KEGG分析，并建立肺癌细胞增殖调控网络模型。

*ZBTB33*基因位于Xq23，编码的Kaiso蛋白属于锌指蛋白超家族中BTB/POZ转录因子亚家族的成员，该蛋白于1999年由Daniel等[14]利用酵母双杂系统研究发现。刘洋等[15]利用转染、Real-Time PCR等方法，发现p120ctn可能通过其分子伴侣Kaiso来上调β-catenin的转录。Kaiso蛋白兼具与序列特异性的DNA结合和与甲基化依赖的基因调节区域结合的功能，而使其在肿瘤的发生发展中发挥着复杂的调控功能。Kaiso蛋白与肺癌的TNM分期较晚和肿瘤淋巴结转移密切相关，并且Kaiso蛋白的表达是非

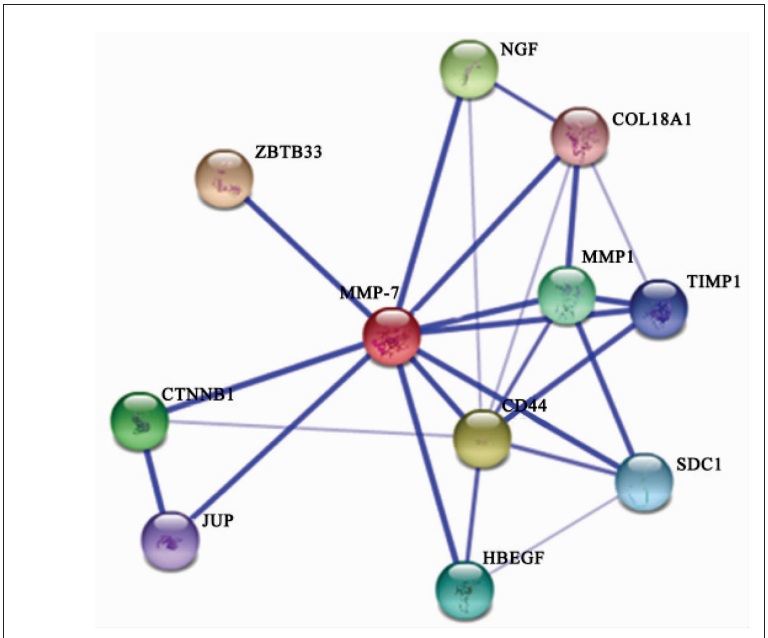

Figure 2　MMP-7 protein cross-linking analysis[13]

小细胞肺癌预后的独立危险因素[16]。COL18A1 蛋白能够产生内皮抑制素，内皮抑制素能够与细胞核表面的核仁蛋白结合，进而抑制核仁蛋白的磷酸化，引起内皮细胞生长周期的阻滞和凋亡，最终影响内皮细胞的增殖及迁移作用，并且内皮抑制素这种抑制功能对血管内皮细胞具有特异性，但目前 COL18A1 产生内皮抑制素的机制尚不清楚[17~20]。

神经生长因子(nerve growth factor，NGF)是一种含量很低能够促进和维持特异性神经元生长、存活和分化的可溶性多肽因子，是最早发现的神经营养因子家族成员之一，并与多种肿瘤的发生、发展密切相关。NGF 对肿瘤细胞的增殖和侵袭具有肿瘤特异性，NGF 对非小细胞肺癌、甲状腺乳头状囊腺癌等肿瘤细胞具有抗增殖和抗侵袭的作用，NGF 则在乳腺癌、前列腺癌、胰腺癌等肿瘤细胞中具有刺激肿瘤细胞的增殖和促进侵袭的作用[21]。肝素结合表皮生长因子样生长因子(heparin-binding epidermal growth factor-like growth factor，HBEGF)是表皮生长因子家族成员之一，HBEGF 可与 EGF 受体结合形成，启动细胞间信号传递，可促进细胞的生长和增殖，并与银屑病表皮异常增殖有关[22]，而 Pro HBEGF 可能诱导肿瘤细胞的凋亡，并与其他抑癌基因共同作用，抑制肿瘤的发生、发展[23]。刘欣欣等[24]研究表明，HBEGF 蛋白的产生可能与 MAPK-ERK1/2 和 JAK2/STAT3 两条信号通路有关。王国荣等[25]利用免疫组织化学和原位杂交的方法发现，在胃癌的整个发展过程中，HBEGF 的表达量逐渐升高，HBEGF 的产生有利于促进肿瘤细胞的增殖，进而促进肿瘤的发生、发展。

组织金属蛋白酶抑制剂(tissue inhibitor of metalloproteinanse，TIMPs) 是 MMPs 家族的抑制剂，TIMPs 和 MMPs 共同调控 ECM 的更新以维持细胞的稳定。TIMP1 由巨噬细胞和结缔组织细胞产生的一种糖蛋白，广泛存在于组织和体液中，可抑制包括 MMP-1 在内的所有胶原酶。一般情况下，在恶性组织中 MMPs 和 TIMPs 表达往往处于平衡状态，当 MMPs 的表达高于 TIMPs 时，平衡遭到破坏，最终导致肿瘤的侵袭和转移 [26]。彭再梅等 [27] 研究发现，MMP-1 和 TIMP1 蛋白的表达与肺癌的发生发展、生物学行为、侵袭和转移有关，并且 MMP-1 和 TIMP1 可作为重要的生物学标记物。*CTNNB1* 基因编码 β-catenin 蛋白，β-catenin 蛋白在细胞核、细胞质和细胞核中均有表达，在细胞质中 β-catenin 蛋白可作为 Wnt 信号通路中的重要信号，参与下游多种基因的表达调控，可导致细胞的恶性增殖分化，最终导致肿瘤的发生、发展、侵袭和转移。研究表明 β-catenin 蛋白在细胞核和细胞质中的表达与非小细胞肺癌的病理分期有关，可作为非小细胞肺癌患者预后指标之一，而且 β-catenin 蛋白在细胞核和细胞质中表达的患者 5 年生存期显著低于未表达的患者[28]。

JUP 基因编码 cGMP 依赖的蛋白激酶(cGMP dependent kinase，PKG)，该蛋白能够调控肿瘤细胞内核心蛋白的表达，在肿瘤细胞的增殖及凋亡过程中发挥着重要的作用 [29]，PKG 蛋白参与 ras 家族蛋白、多种生长因子受体、细胞骨架蛋白的磷酸化[30]，与多种肿瘤细胞的发生、发展密切相关。CD44 分子属于细胞黏附分子家族，广泛存在于多种细胞组织及细胞表面，在多种肿瘤中高表达，并与肿瘤的生长、侵袭、转移等密切相关，是多种肿瘤干细胞的标志物。研究表明，CD44 表达量降低可以阻滞使细胞周期，从而抑制细胞的增殖；CD44 表达量降低可以诱导 β-catenin 的磷酸化水平增加而抑制细胞的增殖[31]。多配体蛋白聚糖 1(syndecan 1，SDC1)是跨膜蛋白聚糖家族成员之一，参与细胞的增殖、粘附、迁移、和血管生成等过程，也是某些肿瘤预后的指标之一[32]。石爽等[33]通过 qRT-PCR 和 Western Blotting 方法，发现沉默 *SDC1* 基因的表达可以抑制胶质瘤 A172 细胞的增殖、迁移和侵袭，SDC1 可以作为胶质瘤生物治疗的新靶位点。王丽等[34]在对甲状腺微小乳头状癌中的研究中发现 SDC1 可能与微小癌的侵袭、转移中发挥重要作用。

综上所述，与 MMP-7 有关联作用的 ZBTB33、NGF、COL18A1、MMP-1、TIMP1、CTNNB1、JUP、CD44、SDC1、HBEGF 均与肺癌细胞增殖有密切联系，本研究通过初步建立的肺癌细胞增殖调控网络，为肺癌的靶向治疗提供一定的理论依据，随着以后研究的不断深入，经过对肺癌调控网络不断进行补充完善，逐步实现肺癌的个性化治疗。

参考文献：

[1] Yao XJ，Liu LX. Epidemiology and treatment status of lung cancer [J]. J Mod Oncol，2014，22(8)：1982-1986. [姚晓军，刘伦旭. 肺癌的流行病学及治疗现状[J]. 现代肿

瘤医学,2014,22(8):1982-1986.]
[2] Wood DE,Eapen GA,Ettinger DS,et al. Lung cancer screening[J]. J Natl Compr Canc Netw,2012,10(2):240-265.
[3] Ueno H,Yamashita K,Azumano I,et al. Enhanced production and activation of matrix metalloproteinase-7 (matrilysin) in human endometrial carcinomas[J]. Int J Cancer,1999,84(5):470-477.
[4] Wroblewski LE,Noble PJM,Pagliocca A,et al. Stimulation of MMP-7 (matrilysin) by Helicobacter pylori in human gastric epithelial cells:role in epithelial cell migration[J]. J Cell Sci,2003,116(14):3017-3026.
[5] Kioi M,Yamamoto K,Higashi S,et al. Matrilysin (MMP-7) induces homotypic adhesion of human colon cancer cells and enhances their metastatic potential in nude mouse model[J]. Oncogene,2003,22(54):8662-8670.
[6] Tanioka Y,Yoshida T,Yagawa T,et al. Matrix metalloproteinase-7 and matrix metalloproteinase-9 are associated with unfavourable prognosis in superficial oesophageal cancer[J]. Br J Cancer,2003,89(11):2116-2121.
[7] Li TQ. Bioinformatic analysis and functional prediction of prostate cancer metastasis related genes [D]. Guangzhou:Southern Medical University,2014. [李铁求. 前列腺癌转移相关基因的生物信息学分析及功能预测[D]. 广州:南方医科大学,2014.]
[8] Sutnar A,Pesta M,Liska V,et al. Clinical relevance of the expression of mRNA of MMP-7,MMP-9,TIMP-1,TIMP-2 and CEA tissue samples from colorectal liver metastases [J]. Tumor Biol,2007,28(5):247-252.
[9] Zucker S,Wang M,Sparano JA,et al. Plasma matrix metalloproteinases 7 and 9 in patients with metastatic breast cancer treated with marimastat or placebo:Eastern Cooperative Oncology Group trial E2196 [J]. Clin Breast Cancer,2006,6(6):525-529.
[10] Yoshida H,Sumi T,Hyun Y,et al. Expression of survivin and matrix metalloproteinases in adenocarcinoma and squamous cell carcinoma of the uterine cervix[J]. Oncol Rep,2003,10(1):45-49.
[11] Duan GJ,Yan XC,Bian XW,et al. The significance of β-catenin and matrix metallopopteinase-7 expression in colorectal adenoma and carcinoma [J]. Chin J Pathol,2004,33(6):518-522. [段光杰,阎晓初,卞修武,等. β-链接素和基质金属蛋白酶-7 在结直肠腺瘤一腺癌组织表达的意义[J]. 中华病理学杂志,2004,33(6):518-522.]
[12] Busiek DF,Baragi V,Nehring LC,et al. Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones[J]. J Immunol,1995,154(12):6484-6491.
[13] Li L,Wang LX,Xu GL,et al. Bio-informatics analysis of renal carcinoma gene matrix metalloproteinase-7[J]. Indian J Cancer,2016,53(1):13-18.
[14] Daniel JM,Reynolds AB. The catenin p120 ctn interacts with Kaiso,a novel BTB/POZ domain zinc finger transcription factor[J]. Mol Cell Biol,1999,19(5):3614-3623.
[15] Liu Y,Miao Y,Han Q,et al. p120-catenin regulates β-catenin expression in lung cancer LTEP-a-2 [J]. J Mod Oncol,2016,24(12):1845-1850. [刘洋,苗原,韩强,等. p120-catenin 通过 Kaiso 在转录水平上调控肺癌细胞系 LTEP-a-2 中 β-catenin 的表达 [J]. 现代肿瘤医学,2016,24(12):1845-1850.]
[16] Wang B. Expression of Kaiso protein in non-small lung cancer tissue and prognosis evaluation [D]. Jilin:Jilin University,2014. [王宝. Kaiso 蛋白在非小细胞肺癌组织中的表达及对预后评估相关性研究[D]. 吉林:吉林大学,2014.]
[17] Wen W,Moses M A,Wiederschain D,et al. The generation of endostatin is mediated by elastase[J]. Cancer Res,1999,59(24):6052-6056.
[18] John H,Radtke K,Ständker L,et al. Identification and characterization of novel endogenous proteolytic forms of the human angiogenesis inhibitors restin and endostatin[J]. Biochim Biophys Acta,2005,1747(2):161-170.
[19] Schmidt A,Wenzel D,Ferring I,et al. Influence of endostatin on embryonic vasculo - and angiogenesis [J]. Dev Dyn,2004,230(3):468-480.
[20] Shi H,Huang Y,Zhou H,et al. Nucleolin is a receptor that mediates antiangiogenic and antitumor activity of endostatin[J]. Blood,2007,110(8):2899-2906.
[21] Meng LX. Molecualr mechanisms study of β-NGF and its receptors on the effects of perineural invasion of pancreatic adenocarcinoma [D]. Tianjin:Tianjin Medical University,2008. [孟令新. β-NGF 及受体在胰腺癌嗜神经性中的分子机制研究[D]. 天津:天津医科大学,2008.]
[22] Yoshida A,Kanno H,Watabe D,et al. The role of heparin-binding EGF-like growth factor and amphiregulin in the epidermal proliferation of psoriasis in cooperation with TNFα[J]. Arch Dermatol Res,2008,300(1):37-45.
[23] Kramer C,Klasmeyer K,Kikuchi K,et al. Heparin-binding epidermal growth factor-like growth factor isoforms and epidermal growth factor receptor/ErbBl expression in bladder cancer and their relation to clinical outcome[J]. Cancer,2007,109(10):2016-2024.
[24] Liu XX,Luo SJ,Zheng Y,et al. Mechanisms underlying interleukin-22-induced expression of heparin-binding epi-

dermal growth factor-like growth factor in HaCaT cells [J]. Chin J Dermatol,2015,48(003):179–183. [刘欣欣,罗素菊,郑焱,等. 白细胞介素 22 诱导 HaCaT 细胞表达肝素结合表皮生长因子样生长因子的作用机制探讨[J]. 中华皮肤科杂志,2015,48(003):179–183.]

[25] Wang GR,Zheng Y,Che XM,et al. Expression of heparin-binding epidermal growth factor-like growth in patients with gastric carcinoma [J]. J Southern Med Univ,2009,29(1):44–46. [王国荣,郑焱,车向明,等. 胃癌中 HB-EGF 的表达及意义[J]. 南方医科大学学报,2009,29(1):44–46.]

[26] Zhang XY. Expression and clinical significance of MMP1, TIMP1 and TIMP3 in laryngeal squamous cell carcinoma [D]. Shijiazhuang:Hebei Med Univ,2009. [张笑盈. MMP1,TIMP1 和 TIMP3 在喉鳞状细胞癌中的表达及临床意义[D]. 石家庄:河北医科大学,2009.]

[27] Peng ZM,Yang ZL,Liu YW,et al. Expression of MMP1 and TIMP1 proteins in lung cancer and its biological significance [J]. J Hunan Med Univ,2002,27 (2):159–161. [彭再梅,杨竹林,刘友文,等. MMP1,TIMP1 在肺癌组织中的表达及临床生物学意义[J]. 湖南医科大学学报,2002,27(2):159–161.]

[28] Li XQ. Nuclear β-catenin accumulation is associated with increased expression of Nanog protein and predicts poor prognosis of non-small cell lung cancer[D]. Guangzhou: Southern Medical University,2013. [李锡清. 细胞核中 β-catenin 和 Nanog 在非小细胞肺癌中的表达及预后相关分析[D]. 广州:南方医科大学,2013.]

[29] Bassi DE,De Cicco RL,Cenna J,et al. PACE4 expression in mouse basal keratinocytes results in basement membrane disruption and acceleration of tumor progression [J]. Cancer Res,2005,65(16):7310–7319.

[30] Seidah NG. The proprotein convertases,20 years later[J]. Methods Mol Biol,2011,768:23–57.

[31] Chang GQ. Biological effects of CD44 in tumor [D]. Beijing:Chinese Academy of Medical Sciences & Peking Union Medical College,Peking Union Medical College; Peking Union Medical College,Tsinghua Uninversity, Chinese Academy of Medical Sciences,2013. [常国强. CD44 在肿瘤中的生物学功能研究[D]. 北京:北京协和医学院中国医学科学院,北京协和医学院,清华大学医学部,中国医学科学院,2013.]

[32] Wang P. Detection and significance of SDC-1,HLA-G and MMP-9 in endometriosis [D]. Zhengzhou:Zhengzhou University,2014. [王平. SDC-1,HLA-G 和 MMP-9 在子宫内膜异位症中的检测及其意义[D]. 郑州:郑州大学,2014.]

[33] Shi S,Zhong D,Wang B,et al. Syndecan-1 knockdown inhibits the proliferation and invasion of A172 glioblastoma multiforme cells [J]. Chin J Nervous Mental Dis,2016,42(2):74–79. [石爽,钟东,王兵,等. Syndecan-1 基因沉默抑制胶质瘤 A172 细胞增殖和侵袭[J]. 中国神经精神疾病杂志,2016,42(2):74–79.]

[34] Wang L,Dong JM,Luo JF,et al. Expression of syndecan-1 and EGFR in papillary thyroid microcarcinoma [J]. Chin J Diagn Pathol,2015,22(4):221–224. [王丽,董军明,罗金芳,等. 甲状腺微小乳头状癌中 syndecan-1 和 EGFR 的表达[J]. 诊断病理学杂志,2015,22(4):221–224.]